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The Root Cause of Post-Traumatic and Developmental Stress Disorder•

PRINCIPAL INVESTIGATOR:
Keith A. Young, PhD

CONTRACTING ORGANIZATION:
Texas A&M Health Science Center

Temple Texas 76504

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14. ABSTRACT Our overarching scientific hypothesis holds that serotonergic influences on brain development, driven by genetics and early experience, induce a variation of normal brain anatomy that makes the brain highly susceptible to the effects of severe stress. After TATRC review in January of 2011, a revised research plan was developed to include a pre/post-deployment study at Fort Hood and anatomical studies of PTSD in collaboration with NIMH, Yale and USUHS. BAMC IRB for the clinical study was approved in December 2012 and the study was approved by HRPO in October 2013, with no changes recommended by HRPO. However, BAMC review of changes in the protocol has further delayed BAMC approval. Post-mortem brain tissue from 30 PTSD, 30 MDD and 30 controls is being studied with several molecular approaches. A subgroup of Control and PTSD cases (N=8) is being studied with anatomical and molecular techniques. Initial golgi analysis of prefrontal anatomy and stereological studies of the frontal cortex in Nissl sections are in progress. Major findings being prepared for publication include 1) Decreased mature dendritic spine density in the straight gyrus of PTSD BA11 (medio-orbital frontal cortex = mOFCtx) involving mushroom spines, 2) Increased density of stubby spines, suggesting that some mature mushroom spines have regressed to a more immature phenotype in PTSD, 3) evidence of major disruption of microRNA levels in suicide and major depression, 4) Approximately 1 in 6 microRNAs (112/716) are elevated in medial straight gyrus in PTSD, indicating a major change in cell physiology, 5) Considering that BA11 is a vital link between the limbic system and brainstem centers governing HPA activity, heart rate, startle and other physiological systems that are dysregulated in PTSD, this data suggests that anatomical and synaptic irregularities in BA11 may underlie a loss of synaptic plasticity that normally allows this region to gate physiological drive into brainstem, resulting in HPA axis dysregulation and psychophysiological distress. Additional analysis suggests that these changes may be driven by hematopoietic factors that are disrupted in PTSD.					
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Table of Contents

	<u>Page</u>
Introduction.....	4
Body.....	5
Key Research Accomplishments.....	5
Reportable Outcomes.....	7
Conclusion.....	7
References.....	N/A
Appendices.....	8

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INTRODUCTION:

This research has been funded in two installments (Phase I and Phase II contracts). The research described below is continuing through 2016 with implementation of the phase II contract, when the main body of the data will be available for analysis and publication.

Our overarching scientific hypothesis holds that serotonergic influences on brain development driven by genetics and early experience induce a variation of normal brain anatomy that makes the brain highly susceptible to the effects of severe stress. The goal of Project 1 is to describe the progression of post-deployment stress disorders (PTSD, major depression, suicidality) in active duty troops using predeployment/postdeployment structured clinical interviews, and to investigate developmental and environmental factors that influence predisposition to PTSD and depression. A subset of participants will be selected to have predeployment/postdeployment MRI and psychophysiological analysis. Using DNA gathered from volunteers, we will investigate genetic factors influencing resiliency and susceptibility to stress disorders using a panel of 20 genes that we have tested and validated. Project 2 will investigate post-mortem anatomy in subjects with major depression and/or PTSD. Both molecular and anatomical techniques will be employed to study the brains already collected. An overarching goal of the Program is integration of data across the projects to compare and contrast the potential for different assessment paradigms (MRI anatomy, fMRI, evoked potentials, startle, genetic profiling) to screen for resiliency and predisposition to post-traumatic and developmental stress disorder stress disorders.

BODY:

KEY RESEARCH ACCOMPLISHMENTS:

Administrative:

Work on the post-mortem portion of the proposal have progressed at accelerated pace, as described below. The IRB for Project 1 has completed initial review at BAMC and has received HRPO approval. We are awaiting personnel and other changes before starting recruitment, Initial HRPO approval was received in October 2013 and we are preparing revisions needed to implement the study, due to changes in procedures over the past 1.8 years when the project was originally submitted. The human subjects portion of the proposal has not been initiated because of additional changes required by BAMC IRB. The budget transition at TAMHSC from the Phase 1 to Phase II funding to support the research was finally completed last month (after a delay of three years) with the assistance of Ms Wahl, in October 2014. As reported to "MOMRP PTSD Biomarkers IPR" at Fort Detrick in February, 2014, the human subjects administrative issues for the project continues to be problematic. That being noted, the scientific aims of the original proposal have been accomplished by other means of funding. In this report, we present evidence for a new .

Project Specific:

Project 1: Longitudinal study

Task 1: Sample 2000 active duty/guard troops predeployment

- a. Diagnostic interview (SCID)
 - b. Depression symptoms
- c. Stress battery (DRRI, development history, suicidality)
 - d. Blood for DNA/RNA
 - e. Medical testing (CBC/TSH/CMP)

Task 2: Resample/test post-deployment

Initial IRB review is completed at BAMC and initial approval from HRPO has been received. 3 resubmissions to BAMC IRB have been made to recalibrate the protocol to current standards for research at Fort Hood, but problems have been encountered in receiving approval for items such as implementing eCRF (electronic Case Report Forms) procedures.

Project 2 Neurobiology

Task 1: Pre-deployment/post-deployment MRI testing 300 scanning sessions

The MRI is still being repaired.

Task 2. Collection of PTSD, MDD and control brains

We have been wildly successful in preparing and analyzing PTSD brains. A total of 30 PTSD, 30 MDD (psychiatric controls) and 60 normal controls have been diagnosed and comprise our current cohort, the largest PTSD post-mortem cohort in existence (only one post-mortem study in PTSD has been published by another group, N= 6 PTSD, 6 control). Initial studies on this tissue is underway as described below and we have prepared one publication on a subset of the sample. Additional specimens continue to be accrued into the collection.

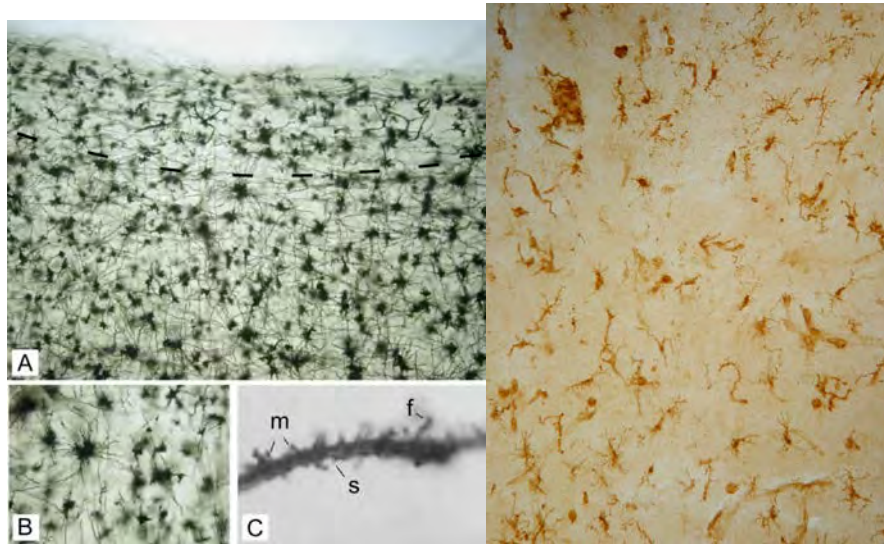
Task 3. Compare gene expression in the frontal cortex of PTSD and controls.

Sample set 1) Frontal cortical tissue (area 9/25) from 23 PTSD (Appendix), 25 MDD and 25 controls have completed gene expression and methylation procedures. RNAseq analysis will be performed on these samples starting in the next 6 months. miRNA sequencing has been completed and the data are being aligned. Data cleaning and analysis are being performed at this time and we expect initial publications within 6 months.

Sample set 2) In the medial orbitofrontal cortex in a subset of these brains (N=8 PTSD and 8 controls), we have performed gene expression, gene methylation and microarray studies which are in analysis (see task 4 below).

Task 4. Compare anatomical markers in frontal cortex/hippocampus of PTSD, MDD and controls, with 5HTTLPR and other genetic variants as cofactors.

Fig A. Golgi staining (left) in the mOFCtx has been performed on 8 PTSD and 8 Control specimens.



We have submitted a paper concluding that there is an increased stubby spine density in BA11 (mOFCtx) and a trend for decreased mushroom spine density in PTSD (BA11), consistent with animal models. A subset of mature mushroom spines appear to have been partially replaced by stubby spines. Stubby spines are less sophisticated than mushroom spines because they do not have necks, where modulatory neurotransmission can be applied to alter excitatory glutamatergic activity. This is the first confirmation that changes in dendritic spines may be a “final common pathway” for extreme stress in humans, as has been observed in animal models. Considering that BA11 is a vital link between the limbic system and brainstem centers governing HPA activity, heart rate, startle and other physiological systems that are dysregulated in PTSD, our data suggest that synaptic irregularities in BA11 may underlie a loss of synaptic plasticity that normally allows this region to gate physiological drive into the brainstem, resulting in HPA axis dysregulation and psychophysiological distress.

In the final quarter of this year, we continued analysis of gene expression correlates of changes in BA11 spine density. Initially, we observed that these changes might be related to Alzheimer’s-related genes, however, the initial changes were largely driven by a single outlier datapoint (as reported in Q3). On re-analysis, the data now suggest that alterations in brain immune pathways may underlie some of the changes in PTSD including spine density changes. It is notable that FKBP5, an immunophilin protein that has previously been linked to PTSD, is one of the top gene transcripts identified in our analysis (Figure B). As we observed in the present study, FKBP5 has been previously observed to be elevated in MDD brain. (Of note, this is opposite of the FKBP5 effect direction observed in blood, where FKBP5 is reduced in PTSD. However, this type of inverse correlation has been observed for other biomarkers when comparing brain and blood). In addition, the re-analysis found several immune-related pathways that (contrary to expectations) are suppressed, rather than activated in PTSD, such as HLA (Figure C).

Fig B: Immunophillin FKBP5 elevated in PTSD BA11 (P<0.023) and correlated to mushroom spine density

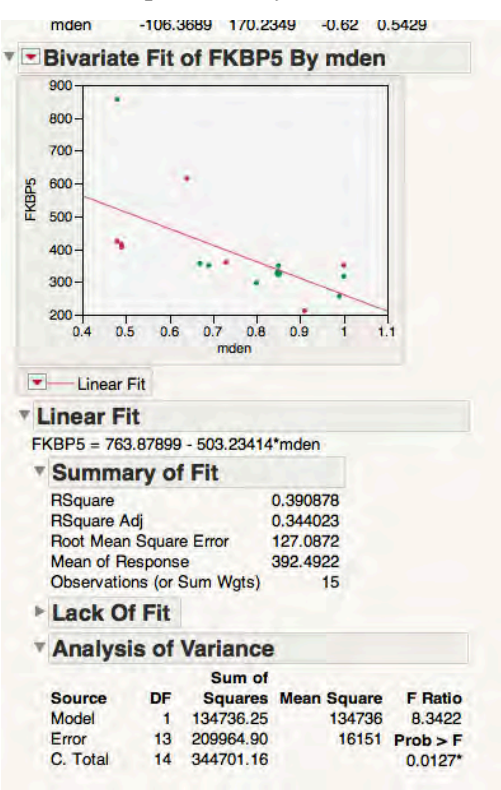
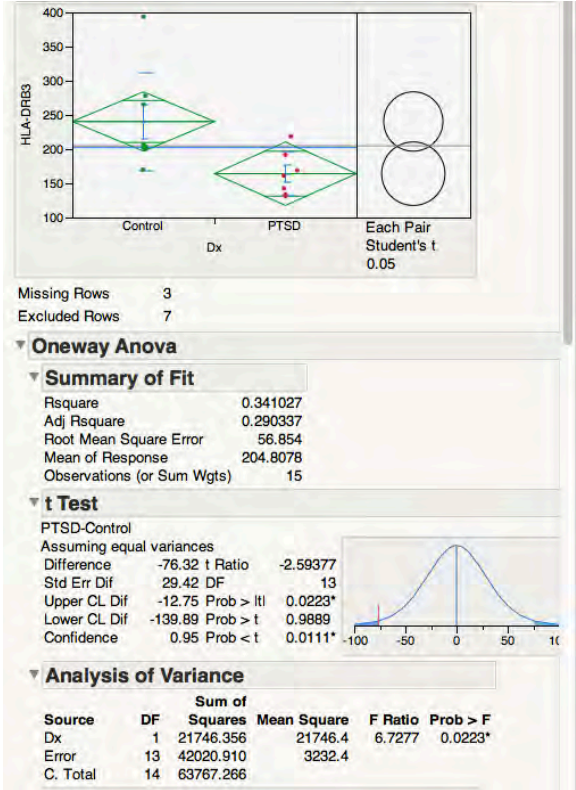


Fig C: Immune-related transcript repressed in PTSD BA11 HLA-DRB3



Nissl staining on whole hemisphere sections from the frontal cortex of PTSD vs controls is being completed in phase II of the study. An initial neuron and glial cell counting study on the straight gyrus of the mOFCtx has been performed.

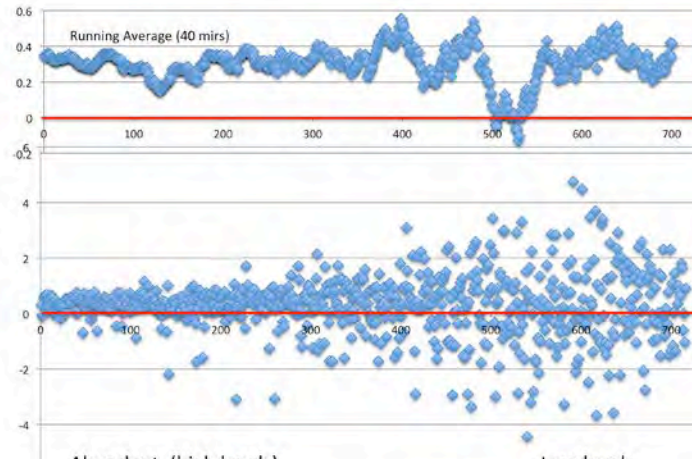
Initial analysis of BA11 indicates that densities of neurons, astrocytes and oligodendrocytes are not significantly affected in PTSD. We are recounting the sections to specifically target microglia, which were not counted in the first pass. In a pilot study, additional sections of BA11 are being stained with specific microglial markers to identify whether there are changes in microglial activation state (90% complete). In fact, microglial activation in our sample appears to be correlated inversely to several measures of PTSD pathophysiology, such as FKBP5 (P<0.0066). These observations suggest the hypothesis that abnormal microglial function may underlie some of the pathophysiology of PTSD. Specifically, since microglial activity is necessary for normal neuronal function, abnormally suppressed microglial activity may be detrimental to synaptic plasticity.



Molecular studies of microRNAs (miRs) in the medial straight gyrus indicates links to PTSD.

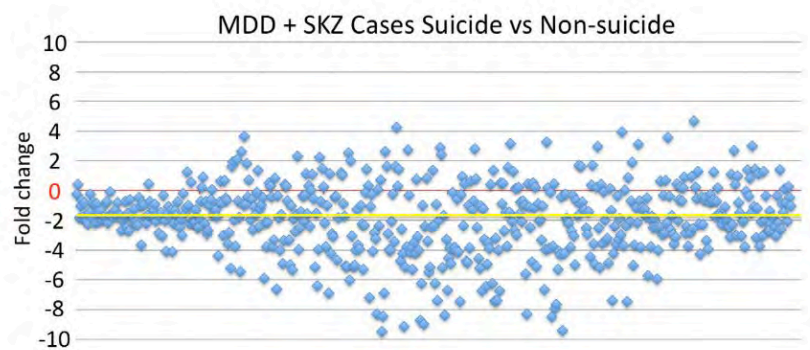
We have performed MiRNA studies of BA11 in PTSD and found that approximately 1 in every 6 (112/716) brain-expressed miRs were significantly elevated in PTSD at a nominal p value of 0.05, while only 4 were significantly decreased. This is strong evidence ($t < 4.2 \times 10^{-17}$, fisher's exact test) for a global up-regulation of miRs in PTSD. Unlike MDD, we found that this increase was not linked to changes in DICER transcript levels.

92 out the 100 most abundant miRs in Cortex are numerically elevated in PTSD ($P < 3.3 \times 10^{-11}$)



MicroRNAs and suicide

We have found that MicroRNA (miR) levels from the mediodorsal thalamus (MDT) are reduced by 2-3 fold in a coordinated manner in MDT tissue from individuals who died by suicide (manuscript in revision). This suggests involvement of microRNA biosynthesis in suicide. This paper is in review.



REPORTABLE OUTCOMES:

None this quarter

CONCLUSION: No scientific conclusions have been made at this point in time.